Draft

Report on Carcinogens Background Document for

2-Amino-3-methylimidazo [4,5-*f*]quinoline (IQ)

Meeting of the
NTP Board of Scientific Counselors
Report on Carcinogens Subcommittee

Prepared for the:
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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

US Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Summary Statement

2-Amino-3-methylimidazo[4,5-*f*]quinoline, IQ

CASRN 76180-96-6

Carcinogenicity

2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ), is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of benign and malignant tumor formation at multiple tissue sites in multiple species of experimental animals (IARC 1993). Oral exposure of rats to IQ induces neoplasms of the mammary gland, liver, small intestine, clitoral gland, oral cavity and Zymbal gland in females and neoplasms of the liver, skin, colon, small intestine, oral cavity and Zymbal gland in males. Oral exposure of mice to IQ induces increased incidences of neoplasms of the lung, liver, and forestomach in males and females. Intraperitoneal exposure to mice and oral exposure to cynomologus monkeys causes liver tumors.

Epidemiology studies have provided some indication that human cancer risk is related to consumption of broiled or fried foods, but there is inadequate evidence that human cancer risk is specifically associated with exposure to IQ or other heterocyclic amines.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Studies of the genotoxicity of IQ have given uniformly positive results in a wide variety of bacterial, plant, and animal assays, predominately in systems providing metabolic activation (IARC 1993). IQ induces mutations, chromosomal aberrations, sister chromatid exchanges, micronuclei and unscheduled DNA synthesis in various human cells in culture. Metabolic activation of IQ to reactive intermediates involves acetylation and hydroxylation. *N*-acetoxy-IQ degrades to an unstable nitrenium ion that can bind to DNA. IQ-DNA adducts have been demonstrated in many tissues in animals receiving IQ, including those where IQ-induced tumors occur. All animal species studied have been found to metabolize IQ to DNA-reactive products, and cells from human mammary gland and microsomes from human liver have been shown to accomplish these reactions *in vitro*.

No data are available that would suggest that the mechanisms thought to account for tumor induction by IQ in experimental animals also would not operate in humans.

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1 Introduction

2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) was nominated for listing in the Report on Carcinogens by the National Institute of Environmental Health Sciences (NIEHS) Report on Carcinogens (RoC) Review Group (RG1) based on review of a 1993 International Agency for Research on Cancer (IARC) monograph which indicated that there is sufficient evidence in experimental animals for the carcinogenicity of IQ and that IQ is *probably carcinogenic to humans* (Group 2A).

1.1 Chemical identification

IQ ($C_{11}H_{10}N_4$, mol wt 198.23, CASRN 76180-96-6) is a light tan, crystalline solid also known as: 3-methyl-3*H*-imidazo[4,5-*f*]quinolin-2-amine. The structure of IQ is illustrated in Figure 1-1.

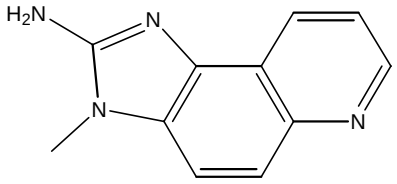

Figure 1-1. Structure of IQ

1.2 Physical-chemical properties

IQ is stable under moderately acidic and alkaline conditions and in cold, dilute aqueous solutions when protected from light (IARC 1986). It is rapidly degraded by dilute hypochlorite (IARC 1993). The RTECS number for IQ is NJ5910000. The physical and chemical properties of IQ are summarized in Table 1-1.

Table 1-1. Physical and chemical properties of IQ

Property	Information	Reference
Molecular weight	198.23	Chemfinder (1999), Budavari *et al.* (1996)
Color	light tan	Chemfinder (1999), Budavari *et al.* (1996)
Physical state	crystalline solid	Chemfinder (1999), Budavari *et al.* (1996)
Melting point (^oC)	> 300	IARC (1993)
Solubility:		
Water at 20°C	insoluble	CSL (1987)
Dimethylsulfoxide	soluble	IARC (1993)
95% Ethanol at 16°C	1 - 10 mg/mL	Radian (1991)
Methanol	soluble	IARC (1993)
Acids (dilute)	soluble	CSL (1987)
Alcohols (dilute)	soluble	CSL (1987)

IQ is one of a number of heterocyclic amines found in cooked food, primarily in meats and fish. IQ has no commercial uses; however, some is synthesized for research purposes, and the majority of the studies reviewed in this document were performed using chemically synthesized IQ.

2 Human Exposure

2.1 Use

IQ has no known commercial uses (Radian 1991, IARC 1993).

2.2 Production

IQ is produced commercially in small quantities for research. Chemical synthesis was first reported by Kasai *et al.* (1980, cited by IARC 1993). 5,6-Diaminoquinoline was reacted with cyanogen bromide, which produced a cyclic intermediate that was converted to the tetramethyl ammonium salt and then heated under reduced pressure to form IQ. Sublimation, silica-gel column chromatography, and crystallization from aqueous methanol were used to purify the IQ (IARC 1993).

2.3 Analysis

IQ is found predominantly in cooked foods. Table 2-1 describes the various methods of analysis for determining concentrations of IQ.

Table 2-1. Analytical methods for determining IQ concentration

Sample matrix	Assay procedure[a]	Preparation	Reference
Broiled, sun-dried sardines	NMR and high-resolution mass spectral analysis	extracted with methanol and purified by Diaion PH-20 column chromatography, chloroform-methanol-water partitioning and Sephadex LH-20 column chromatography, silica-gel column chromatography and reverse-phase HPLC	Kasai *et al.* (1980, 1981)
Beef extract	mass spectrometry and ultraviolet spectrophotometry and/or mutagenic assays	isolated by dichloromethane extraction, column chromatography on Adsorbosil-5 and Sephadex LH-20 and HPLC	Hargraves and Pariza (1983); Turesky *et al.* (1983)
Fried ground beef	off-line mass spectrometry	dichloromethane extraction, chromatography on XAD-2 resin and three different HPLC separations	Felton *et al.* (1984)
Aqueous solutions	HPLC	adsorbed onto cellulose or cotton to which C.I. reactive blue 21 has been covalently bound (blue cotton adsorption technique); eluted with an ammonia-methanol solution	Hayatsu *et al.* (1983)
Food and food extracts	HPLC	solid-phase extraction and subsequent analysis allow IQ determination at a level of 1 ng/g food from only 10 g of food	Gross (1990); Gross and Gruter (1992)

Source: IARC (1993).
[a] NMR: nuclear magnetic resonance; HPLC: high-performance liquid chromatography.

2.4 Environmental occurrence

IQ is one of many heterocyclic amines (HCAs) formed when various meats and fish are cooked. Originally, it was isolated from broiled fish, fried ground beef, and beef extracts. No uses have

been identified that would be expected to lead to the release of IQ into the environment. Environmental occurrence of IQ may arise from food waste and disposal in landfills.

IQ was found when certain compounds were mixed and heated, such as in meat extracts. Meat extracts typically are formed by heating a source of amino acids, reduced sugars, fats, and other ingredients at temperatures greater than 100°C for times sufficient to develop flavor. Aeschbacher *et al*. (1987, cited in Jackson *et al.* 1994) reported that commercial meat extracts can contain up to 100 ng/g of HCAs (including IQ, methyl-IQ, and dimethyl-IQ) (Jackson *et al.* 1994). Creatine and proline heated to 180°C will produce IQ. IQ also was found in mixtures of creatinine and phenylalanine or creatinine, phenylalanine, and glucose, heated to 200°C and in a dry mixture of serine and creatinine heated to 200°C (IARC 1993). Table 2-2 summarizes IQ concentrations in various products.

Table 2-2. Concentrations of IQ in foods and in cigarette smoke condensate

Sample	Concentration wet weight (ng/g)	No. of samples[a]	Reference
Chicken leg, with skin, fried for 15 minutes			
100°C	0.13	10	Chiu *et al.* (1998)
150°C	0.14	10	Chiu *et al.* (1998)
200°C	0.51	10	Chiu *et al.* (1998)
Egg, fried at 325°C	0.1	1	Grose *et al.* (1986)[b]
Fish, fried at 260°C	0.16	1	Zhang *et al.* (1988)[b]
Ground beef, fried			
240°C	0.5 – 20[c]	2	Barnes *et al.* (1983)[b]
250°C	0.02	1	Felton *et al.* (1984)[b]
275°C	0.3 - 1.9	3	Turesky *et al.* (1988)[b]
Ground beef, broiled	0.5	1	Yamaizumi *et al.* (1986)[b]
Beef, broiled	0.19	1	Wakabayashi *et al.* (1992)[b]
Beef extract, food-grade	<0.2	1	Takahashi *et al.* (1985)[b]
Beef extract, food-grade	< 0.1 - 6.2	3	Turesky *et al.* (1989)[b]
Minute steak cooked at 150°C for 2.5 min	0.1	—	Skog *et al.* (1995)
Meat balls cooked at 175°C for 7.5 min	0.05	—	Skog *et al.* (1995)
Pork chops cooked at 175°C for 11 min	0.1	—	Skog *et al.* (1995)
Salmon, broiled	0.3 - 1.8	2	Yamaizumi *et al.* (1986)[b]
Sardines, sun dried, broiled	~ 20	1	Kasai *et al.* (1980)[b]
Sardines, sun dried, broiled	158	1	Sugimura *et al.* (1981)[b]
Cigarette smoke condensate	0.26 per cigarette	—	Yamashita *et al.* (1986)[b]

[a] (—), number of samples not given.
[b] Cited in IARC (1993).
[c] 20 ng/g in a high-fat sample, 0.5 ng/g in a low-fat sample.

2.5 Environmental fate

No environmental fate data could be found. IQ's persistence in the environment after any potential release is unknown.

IQ degrades in light and air. It may be sensitive to prolonged heat exposure. A solution of IQ in water (concentration not specified), dimethylsulfoxide (DMSO), 95% ethanol, or acetone is stable for 24 hours under ambient conditions (Radian 1991).

2.6 Environmental exposure

The most likely mode of exposure is ingestion of cooked food products containing IQ, such as broiled or fried beef, fish, or eggs (Radian 1991). IQ also is present in cigarette smoke (Yamashita *et al.* 1986). Based upon analysis of various foodstuffs and analysis of HCA content (including IQ), estimated daily exposure of the U.S. population to heterocyclic aromatic amines ranges from 100 ng to 10 µg per day. It is difficult to quantify overall U.S. exposure, because IQ content depends on the meat, cooking temperature, and manner of preparation (Turesky *et al.* 1993). Individual IQ intake would depend upon these factors.

Another study estimated human exposure to five heterocyclic amines in an elderly population in Stockholm. After a semi-quantitative food survey was administered, 22 dishes were cooked and chemically analyzed. The total daily dietary intake of the heterocyclic amines ranged from 0 to 1,816 ng, with a mean intake of 160 ng. Of this mean daily HCA intake, IQ intake was < 1 ng/day (Augustsson *et al.* 1997).

2.7 Occupational exposure

Occupational exposure to IQ may occur where employees work with broiled or fried foods, such as beef, fish, or eggs. No studies were found that dealt with IQ intake among workers who prepared or served such foods. It is unknown whether exposure by routes other than ingestion (i.e., dermal or inhalation) would occur in this setting.

2.8 Biological indices of exposure

IQ undergoes metabolic activation to yield the reactive metabolite *N*-acetyl-IQ, which binds to DNA (Probst *et al.* 1992). Using the ^{32}P-postlabeling method, Fan *et al.* (1995) found IQ-DNA adducts in human mammary gland epithelial cells following *in vitro* exposure to IQ. Leong-Morganthaler *et al.* (1998) also found IQ-DNA adducts in TK6 human lymphoblastoid cells following *in vitro* exposure to IQ, using the same method.

Ten urinary IQ metabolites have been identified in animal studies (see Section 6), but no studies demonstrating the occurrence of these metabolites in humans were located.

2.9 Regulations

No IQ-specific regulations were found. One regulation (40 CFR 721) states that significant new uses for substituted quinoline must be reported. Currently, however, IQ has no known commercial uses.

3 Human Cancer Studies

A substantial body of literature suggests that risk of several cancers may be related to consumption of meat, particularly red meat, and to methods of food preparation, particularly grilling and frying (IARC 1993). The mechanism underlying this risk is as yet unclear. One possibility is that HCAs formed when meat is cooked are involved. This section reviews studies that assessed the relationship of human cancer risk to exposure to IQ or to total HCAs. The major problem encountered in these studies was the difficulty of quantifying HCA exposure. The studies are summarized in Table 3-1.

De Stefani *et al.* (1997) conducted a hospital-based case-control study in Uruguay to determine the relationship of IQ exposure to risk of breast cancer in pre- and post-menopausal women. Between May 1994 and November 1996, 352 newly diagnosed breast cancer patients in six major hospitals were enrolled in a case-control study. During the same time period and in the same hospitals, 382 patients with non-neoplastic diseases were enrolled as controls. Controls were frequency-matched to cases by age, sex, and residence. In-person interviews were used to collect information on demographic and anthropometric factors, occupational history, alcohol and tobacco use, and family history of cancer. The interview also included a food-frequency questionnaire, which collected information on consumption of 64 food items over the two years prior to the interview or the onset of symptoms. Data on food consumption and cooking practices were used to estimate total IQ intake, which was then categorized into quartiles. Risks were adjusted for age, residence, family history of breast cancer, age at menarche, parity, history of benign breast disease, total calories, vegetable intake, and fat intake. Considering both pre- and post-menopausal women, odds ratios (ORs) increased from 1.22 (95% CI 0.75 - 1.99) for quartile II to 1.87 (95% CI 1.10 - 3.15) for quartile III and to 3.34 (95% CI 1.85 - 6.02) for quartile IV (P for trend < 0.001). Similar results were seen in post-menopausal women, but the association was weaker in pre-menopausal women, possibly because of the smaller sample size. The strengths of this study are large sample size, ability to account for other breast cancer risk factors, and the attempt to quantify IQ exposure. Limitations include the hospital-based design and difficulties in estimating IQ exposure, including poor recall of usual diet by hospitalized participants and failure to account for smoking as a source of IQ exposure.

Probst-Hensch *et al.* (1997) conducted a sigmoidoscopy-based case-control study in California to determine the relationship of dietary HCA exposure to prevalence of distal colorectal adenomas. A total of 488 individually matched case-control pairs were selected from two southern California medical centers where they had undergone sigmoidoscopy between January 1, 1991, and August 25, 1993. Cases had a first diagnosis of histologically confirmed adenoma. Controls had no current or past polyp of any kind and were individually matched to cases by age, gender, date of sigmoidoscopy, and medical center. In-person interviews were used to collect data on smoking, therapeutic drug use, physical activity, height, weight, and family history of cancer. A semiquantitative food-frequency questionnaire was administered, which used nine categories, ranging from “never or less than once per month” to “six or more a day,” to determine food consumption during the previous year. Percent of time meat was fried was associated with adenoma prevalence (P for trend = 0.004), but degree of doneness, darkening of meat surface, and percent of time meat was barbecued were not. Only total HCA and not IQ exposure was estimated. Two estimates of HCA exposure were made, based on degree of doneness or on

surface darkening of red meat. Participants who ate red meat at most once per week, fried it at most 10% of the time, and preferred meat that was not well done or darkly browned, respectively, were classified as having low exposure. Those who ate red meat more than once per week, who fried it more than 10% of the time, and who preferred it well done or darkly browned, respectively, were classified as having high exposure. Others were classified as having intermediate exposure. After adjustment for smoking, calories, and fruit and vegetable intake, HCA exposure calculated by either method was associated with adenoma prevalence: for doneness, OR for high vs low exposure = 1.7 (95% CI 0.9 - 3.3), *P* for trend = 0.05; for darkening, OR = 2.2 (95% CI 1.1 - 4.3), *P* for trend = 0.05. The strengths of the study include large sample size, selection of cases and controls from the same series of patients, ability to account for other colorectal cancer risk factors, and the attempt to quantify HCA exposure. Limitations include examination of adenomas only in the left side of the colon and difficulties in estimating HCA exposure.

Augustsson *et al.* (1999) conducted a population-based case-control study to assess the relationship of daily intake of total or specific HCAs to the risk of colon, rectum, bladder, and kidney cancers in Sweden. Subjects were identified from the population-based Swedish cancer registry and included 352 with colon cancer, 249 with rectal cancer, 273 with bladder cancer, and 138 with kidney cancer. Controls (553) were randomly selected from a population register and frequency-matched to colon cancer cases by age and sex. A semiquantitative food-frequency questionnaire that included 188 food items was used to determine food consumption for the previous five years. Color photographs were used to determine the degree of surface browning of fried meats. Daily intakes of specific HCAs were estimated based on type of meat or fish eaten, frequency of consumption, portion size, cooking methods, and degree of surface browning. Estimates were based on concentrations of HCAs measured in previous studies of foods cooked in standard ways. Median daily intake of total HCAs was less than 100 ng. The highest daily intake reported by controls was 1,816 ng; seven subjects with cancer reported a higher intake. Intakes were categorized into quintiles, and risks were adjusted for age, sex, and smoking (for bladder and kidney cancer only). For total HCAs, weak associations were observed for bladder and kidney cancer, but no dose response was evident. No association with IQ was observed for any cancer. Strengths of the study include the large sample size, the population-based design, and the use of detailed data on diet coupled with measurements of HCAs in cooked foods to estimate HCA intake. Limitations include the restricted range of HCA intake. The results of this study suggest that low levels of HCA intake are not associated with increased cancer risk, but do not exclude the possibility that very high levels of intake may present a risk.

In summary, evidence relating IQ consumption to cancer risk is limited. Of the studies reviewed here, one suggests a relationship of IQ to breast cancer risk, another suggests a relationship of total HCAs to colorectal cancer risk, and the third provides little evidence for a relationship of IQ or total HCAs to risk of cancer of colon, rectum, bladder, or kidney. Although all studies were well conducted, each was hampered by difficulties in estimating exposure to IQ or total HCAs. Thus, the evidence from human studies regarding the relationship of IQ exposure to cancer risk remains inconclusive.

Table 3-1. Case-control studies of HCA or IQ exposure and human cancer risk

Reference	Population	Exposure	Effects	Potential confounders
De Stefani *et al.* (1997) Uruguay	352 breast cancer cases and 382 controls with non-neoplastic diseases recruited from 6 major hospitals between May 1994 and November 1996	IQ intake estimated using a food-frequency questionnaire and information on cooking practices, and then categorized into quartiles: the lowest quartile is the referent category. quartile I: ≤ 0.42 ng/g quartile II: 0.43 - 0.66 ng/g quartile III: 0.67 - 1.01 ng/g quartile IV: ≥ 1.02 ng/g	**breast cancer OR for all subjects** quartile II: 1.22 (95% CI 0.75 - 1.99, 77 cases) quartile III: 1.87 (95% CI 1.10 - 3.15, 92 cases) quartile IV: 3.34 (95% CI 1.85 - 6.02, 130 cases) *P* for trend < 0.001 **breast cancer OR for premenopausal women** quartile II: 1.86 (95% CI 0.54 6.52, 18 cases) quartile III: 2.70 (95% CI 0.76 - 9.53, 18 cases) quartile IV: 2.15 (95% CI 0.58 - 8.05, 32 cases) *P* for trend = 0.24 **breast cancer OR for postmenopausal women** quartile II: 1.11 (95% CI 0.64 - 1.92, 59 cases) quartile III: 1.75 (95% CI 0.96 - 3.19, 74 cases). quartile IV: 3.80 (95% CI 1.90 - 7.60, 98 cases) *P* for trend < 0.001	risks adjusted for age, residence, family history of breast cancer in a first-degree relative, age at menarche, parity, previous history of benign breast disease, total energy, vegetable intake, and fat intake

Reference	Population	Exposure	Effects	Potential confounders
Probst-Hensch *et al.* (1997) United States	488 individually matched pairs of cases and controls recruited from sigmoidoscopy patients at two California medical centers; cases had first diagnosis of histologically confirmed adenoma; controls were free of past or current polyps.	HCA intake estimated from a semiquantitative food-frequency questionnaire and information on cooking practices; estimates based on degree of doneness or surface darkening of meat, and categorized as low, intermediate, or high exposure; low exposure is the referent category.	**colorectal adenoma OR based on meat doneness:** **intermediate**: 1.4 (95% CI 1.0 - 2.0, 307 cases) **high**: 1.7 (95% CI 0.9 - 3.3, 31 cases) *P* for trend = 0.05 **colorectal adenoma OR based on surface darkening:** **intermediate**: 1.3 (95% CI 0.9 - 1.9, 297 cases) **high**: 2.2 (95% CI 1.1 - 4.3, 37 cases) *P* for trend = 0.02	risks adjusted for total calories, smoking, and fruit and vegetable intake

Reference	Population	Exposure	Effects	Potential confounders
Augustsson *et al.* (1999) Sweden	source population of people in Sweden born between 1918 and 1942, who had a permanent address in Stockholm at least one month between November 1, 1992, and December 31, 1994 352 cases of colon cancer, 249 cases of rectal cancer, 273 cases of bladder cancer, and 138 cases of kidney cancer identified from population-based cancer registry; 553 controls randomly selected from population register and frequency matched to colon cancer cases	semiquantitative food-frequency questionnaire used to determine food consumption 5 years previously; color photographs used to determine degree of surface browning of fried meats; daily intake of IQ estimated based on type of meat or fish eaten, frequency of consumption, portion size, cooking methods, and degree of surface browning; estimates were based on concentrations of IQ measured in previous studies of foods cooked in standard ways; intake categorized into quintiles; quintile 1 is the referent category.	**colon cancer OR**: **quintile 2**: 1.1 (95% CI 0.7 - 1.7) **quintile 3**: 0.8 (95% CI 0.5 - 1.3) **quintile 4**: 1.4 (95% CI 0.9 - 2.1) **quintile 5**: 1.1 (95% CI 0.7 - 1.6) **rectum cancer OR**: **quintile 2**: 0.9 (95% CI 0.5 - 1.4) **quintile 3**: 0.8 (95% CI 0.5 - 1.3) **quintile 4**: 1.4 (95% CI 0.9 - 2.2) **quintile 5**: 0.8 (95% CI 0.5 - 1.3) **bladder cancer OR**: **quintile 2**: 0.9 (95% CI 0.5 - 1.5) **quintile 3**: 1.1 (95% CI 0.7 - 1.9) **quintile 4**: 1.0 (95% CI 0.6 - 1.8) **quintile 5**: 1.1 (95% CI 0.7 - 1.9) **kidney cancer OR**: **quintile 2**: 1.0 (95% CI 0.5 - 1.8) **quintile 3**: 0.6 (95% CI 0.3 - 1.2) **quintile 4**: 1.3 (95% CI 0.7 - 2.3) **quintile 5**: 0.9 (95% CI 0.5 - 1.6)	risks adjusted for age, sex, and smoking (bladder and kidney cancer only)

OR: Odds ratio

4 Studies of Cancer in Experimental Animals

4.1 Orally administered IQ

4.1.1 Studies in mice

IARC reviewed studies of IQ carcinogenicity in experimental animals (IARC 1993). In one of these studies, groups of 40 male and 40 female CDF_1 mice ([BALB/cAnN x DBA/2N]F_1), seven weeks of age, were fed either basal diet or diet containing IQ (> 99.6% pure) at a concentration of 300 ppm for 675 days. Survival of animals administered IQ was similar to that of controls. Body weights of females receiving IQ were slightly less than those of controls. Administration of IQ caused significant increases in the incidences of hepatocellular adenomas and carcinomas (combined), adenomas and adenocarcinomas (combined) of the lung, and papillomas and squamous cell carcinomas (combined) of the forestomach in both sexes (Ohgaki *et al.* 1984, 1986, cited in IARC 1993). Table 4-1 presents data from this study.

Table 4-1. Tumor incidences in CDF_1 mice fed a diet containing IQ at a concentration of 300 ppm for up to 675 days

Tumor type	Tumor incidence/number examined			
	Males		Females	
	Control	IQ treated	Control	IQ treated
Liver: adenoma	2/33	8/39	0/38	5/36
carcinoma	0/33	8/39	0/38	22/36**
adenoma and carcinoma (combined)[a]	2/33	16/39*	0/38	27/36**
Lung: adenoma	4/33	13/39	3/38	7/36
adenocarcinoma	3/33	14/39	4/38	8/36
adenoma or carcinoma	7/33	27/39**	7/38	15/36*
Forestomach: papilloma	1/33	11/39	0/38	8/36
squamous cell carcinoma	0/33	5/39	0/38	3/36
papilloma or carcinoma	1/33	16/39*	0/38	11/36*

Source: Ohgaki *et al.* (1984 1986, cited in IARC 1993).
[a]Statistics calculated for combined tumors.
*$P < 0.05$; **$P < 0.01$.

Tudek *et al.* (1989, cited in IARC 1993) administered IQ by gavage to groups of 10 or more female CDF_1 mice, 27 to 31 days old, at doses of 200 or 400 mg/kg bw, one-half the LD_{50}, twice at a four-day interval. The numbers of aberrant colonic crypts, considered a precursor lesion for colon carcinogenesis, were increased in a dose-related manner 21 days after the initial IQ dose. Crypts were found most frequently in the cecal end of the colon.

4.1.2 Studies in rats

A group of 32 female Sprague-Dawley rats, six weeks old, received IQ hydrochloride by gavage at a dose of 0.35 mmol body weight (70 mg/kg) in 5% Emulphor. The dosing regimen was three doses per week during weeks 1 through 4, two doses per week during weeks 5 through 8, and

one dose per week during weeks 9 through 31. Animals were maintained without further dosing until study termination at week 52. Vehicle-control rats were dosed on the same schedule, and a group of nine animals served as untreated controls. Administration of IQ caused increased incidences of mammary gland tumors (14/32); primarily adenocarcinomas, neoplastic nodules (3/32), hepatocellular carcinomas (2/32), and hemangioendotheliomas of the liver (2/32), and squamous cell carcinomas of the Zymbal gland (11/32). No tumors of the mammary gland, Zymbal gland, or liver were observed in control animals (Tanaka *et al.* 1985, cited in IARC 1993*).*

Groups of 40 male and 40 female Fischer 344 rats, eight weeks of age, were fed pelletized diets containing IQ at a concentration of 300 ppm (purity not stated) for 104 weeks. Groups of 50 males and 50 females served as controls. The animals fed IQ had significantly increased incidences of sacrifices in moribund condition and early deaths due to hepatocellular carcinomas, squamous cell carcinomas of the Zymbal gland, and adenocarcinomas of the intestine. One control male was reported to have a hepatocellular carcinoma. No other controls were found to have tumors at any of the sites listed in Table 4-2. A summary of the tumor incidences is presented in Table 4-2 (Takayama *et al.* 1984, Ohgaki *et al.* 1986, both cited in IARC 1993).

Table 4-2. Tumors observed in Fischer 344 rats fed a diet containing IQ at a concentration of 300 ppm for up to 104 weeks

Tumor type	Tumor incidence/number examined			
	Males		Females	
	Control	IQ treated	Control	IQ treated
Zymbal gland: squamous cell carcinoma	0/50	36/40*	0/50	27/40*
Large intestine: adenocarcinoma	0/50	25/40*	0/50	9/40*
Small intestine: adenocarcinoma	0/50	12/40*	0/50	1/40
Liver: hepatocellular carcinoma	1/50	27/40*	0/50	18/40*
Skin: squamous cell carcinoma	0/50	17/40*	0/50	3/40
Oral cavity: squamous cell carcinoma	0/50	2/40	0/50	1/40
Preputial/Clitoral gland: squamous cell carcinoma	NR	NR	0/50	20/40*

Source: Takayama *et al.* (1984), Ohgaki *et al*. (1986), both cited in IARC (1993).
*$P < 0.01$ Fisher's exact test, calculated by NTP (RG1).
NR: not reported

Weisburger *et al.* (1995) investigated the carcinogenicity of IQ in 30 male F344 rats, six weeks old, given IQ by intrarectal infusion at a dose of 35 mmol/kg in DMSO, three times per week for four months, followed by two times per week for 14 more months. Tumor incidences were compared with those in a group of 50 male control rats; unspecified numbers of which were untreated or received the vehicle. Increased incidences of carcinomas of the colon (13/30), squamous cell carcinomas of the skin (11/30), and liver adenomas (5/30) were observed in rats receiving IQ. Controls had incidences of 1/50 for each tumor site and type. In another experiment designed to evaluate the enhancement of the action of IQ by dietary fat, 34 to 35 male and 34 female F344 rats were fed 75 ppm IQ in 5% or 23.5% corn–oil-diets (10% and 40%

of fat calories) for 12 months. Males were sacrificed at 15 months and females at 18 months. Complete necropsies were performed. Increased incidences of tumors, identified as Zymbal gland/earduct neoplasms, multiple sebaceous malignant and benign skin tumors (in males), liver tumors, mammary gland carcinomas, colon tumors, and preputial gland tumors, were observed. Corresponding tumor incidences in groups of 34 controls were reported as 0 or 1. These results are summarized in Table 4-3 (Weisburger *et al.* 1995).

Table 4-3. IQ-induced cancers observed in rats fed 75 ppm IQ in 5% or 23.5% corn-oil-diets

Tumor type	Tumor incidence/corn oil (%)			
	Males[a]		Females[a]	
	5%	23.5%	5%	23.5%
Zymbal gland[b]	14	14	12	4
Lip[b]	5	0	2	3
Skin[b]	16	20	1	0
Liver: carcinoma	2	17	6	6
adenoma	0	5	3	2
Mammary tumors	—	—	10	17
Preputial/clitoral tumors	3	2	9	13
Colon tumors	2	3	0	1
Lung tumors	1	6	1	1
Lung metastases	5[c]	2[d]	0	0

Source: Weisburger *et al.* (1995)
[a] 34 rats examined/group, except 35 in males on 23.5% fat diet; tumor incidence in controls was 0 or 1.
[b] In many cases, these were multiple malignant or benign neoplasms of sebaceous nature.
[c] From sebaceous skin tumors
[d] Carcinomas: one from skin, one from liver.
—, not given.

Tudek *et al.* (1989, cited in IARC 1993) administered IQ by gavage to groups of 10 or more female Sprague-Dawley rats, 21 days old, at doses of 200 or 400 mg/kg body weight, a total of five times at four-day intervals. The numbers of aberrant colonic crypts observed 21 days after the initial IQ dose were dose-related. Crypts were found most frequently in the cecum.

4.1.3 Gavage study in monkeys

Groups of 20 cynomologus monkeys (*Macaca fascicularis*) were administered IQ by gavage in hydroxypropyl cellulose at doses of 10 mg/kg (14 males, 6 females) or 20 mg/kg (8 males, 12 females), five times per week for 60 months. The monkeys were one year old at the initiation of the study. Hepatocellular carcinomas were found in all 20 animals that had received IQ at 20 mg/kg and in 15 animals that had received IQ at 10 mg/kg. Metastases to the lung were observed in several animals. No liver tumors were observed in an unspecified number of control animals from the same colony (Adamson *et al.* 1990, 1991, cited in IARC 1993; Thorgeirsson *et al.* 1996).

4.2 Intraperitoneally administered IQ

Newborn B6C3F$_1$ mice received intraperitoneal injections of IQ (total doses of 0, 0.625, or 1.25 μmol [125 - 250 μg] dissolved in DMSO) on days 1, 8, and 15 after birth. The initial number of mice used in this study was not stated. Animals were sacrificed after 8 and 12 months. The incidence of hepatocellular adenomas was significantly higher ($P < 0.005$) in IQ treated mice than in controls surviving to both sacrifice periods (1/44 controls, 5/24 low-dose mice and 5/16 high-dose mice at 8 months; 5/44 controls, 7/19 low-dose mice and 14/20 high-dose mice at 12 months). Additionally, two hepatocellular carcinomas were found in high-dose animals at 12 months. No carcinomas occurred in controls examined at 8 or 12 months (Dooley *et al.* 1992, cited in IARC 1993).

4.3 Other studies

Numerous investigators have used IQ to induce preneoplastic events or tumors in animals in studies designed to examine the pro- or anti-carcinogenic activities of other substances. Orally administered IQ has been employed to induce hepatic cytochrome P-450 1A-2 (Nerurkar *et al.* 1993; Xu *et al.* 1997); colonic neoplasms, aberrant colonic crypts, and colonic foci of aberrant crypts in rodents (Kristiansen *et al.* 1996; Xu *et al.* 1997; Ferguson and Harris 1998); DNA adducts in rodents (Davis *et al.* 1994; Nerurkar *et al.* 1995, 1996; Turesky *et al.* 1995, 1996a, 1997; Schut *et al.* 1997a, 1997b; Xu *et al.* 1997) and nonhuman primates (Snyderwine *et al.* 1993b; Turesky *et al.* 1997, 1996b); and mammary gland neoplasms in mice (Weisburger *et al.* 1997).

IQ also has been used to induce preneoplastic events or tumors in studies of oncogene mutations in mice (Herzog *et al.* 1993), rats (Makino *et al.* 1992; Kakiuchi *et al.* 1993; Takahashi *et al.* 1993; Makino *et al.* 1994; Tachino *et al.* 1995), and primates (Fujimoto *et al.* 1994). Mutations were detected in colonic tumors and aberrant crypts and in hepatocellular carcinomas, and carcinomas of the Zymbal gland in animals dosed with IQ. In a study in which IQ was used comparatively with 2-amino-1-methyl-6-phenylimidazo-[4,5,-*b*]pyridine to evaluate mutations in induced rat colon tumors, microsatellite instability was increased in the rat colon adenocarcinomas induced by 2-amino-1-methyl-6-phenylimidazo-[4,5,-*b*]pyridine, but not IQ, suggesting impaired DNA mismatch repair (Canzian *et al.* 1994). In other studies, IQ-induced colorectal tumors in F344/N rats showed inhibition of cell death (apoptosis). Colorectal tumors were found to exhibit increased expression of *bcl-2*, an anti-apoptosis protein, and decreased *bax*, an apoptosis activator (Hayashi *et al.* 1996; Dashwood *et al.* 1998). The results of these studies consistently have shown that relatively short periods of administration of IQ cause preneoplastic or neoplastic changes in experimental animals. Mutations in catenins, a tumor suppressor protein, also were noted in IQ-induced colon tumors (Dashwood *et al.* 1998).

4.4 Summary

The carcinogenicity of IQ has been demonstrated in studies with mice, rats, and nonhuman primates. Rats orally exposed to IQ developed neoplasms of the mammary gland, liver, small intestine, skin, oral cavity, preputial/clitoral gland, and Zymbal gland. Mice orally exposed to IQ had increased tumors of the lung, liver, and forestomach. Nonhuman primates administered IQ by gavage developed liver tumors. Intraperitoneal administration of IQ to newborn mice resulted in liver tumors, and intrarectal administration to rats resulted in intestine and liver neoplasia.

5 Genotoxicity

5.1 Prokaryotic Systems

5.1.1 Induction of mutation in Salmonella typhimurium

In studies reviewed by IARC (1993), IQ was consistently found to be mutagenic in a variety of *Salmonella typhimurium* tester strains with metabolic activation.

In more recent studies, IQ induced a significant increase in the induction of *His+* revertants in *S. typhimurium* strain YG1012 (TA1538 1,8-DNP pYG213) in an assay in which human P-450-1A2-containing microsomes and hydrogen peroxide were used as a metabolic activation system. The mutagenic response was found to depend on the concentrations of microsomal proteins, IQ, and hydrogen peroxide. Addition of peroxides greatly enhanced the induction of *His+* revertants (Morrison *et al.* 1993; Anari *et al.* 1997). IQ also was mutagenic in *S. typhimurium* strain TA98 in the presence of metabolic activation provided by microsomal protein (from rat, cynomologus monkey, or human liver) and NADPH. No statistically significant differences in species activation potential were noted (Davis *et al.* 1993).

Coincubation of IQ and ram seminal vesicle microsomes (RSVM), metabolically activated by prostaglandin H synthase and supplemented with arachidonic acid, with *S. typhimurium* strain YG1024 (TA98 pYG219) yielded a dose-dependent increase in the frequency of revertants. Omission of the arachidonic acid supplement inhibited the mutagenicity of IQ. Heating of the RSVM abolished the mutagenicity of IQ in this test system (Wolz *et al.* 1995).

IQ induced a significant dose-dependent increase in base-pair substitution mutations at the *his*G46 allele in *S. typhimurium* strain TA100 in the presence of exogenous rat-liver S9 metabolic activation. The observed mutations were predominantly GC→TA transversions with a pronounced preference for the second codon position, CCC→CAC. IQ was, however, not mutagenic in *S. typhimurium* strain TA1535 in the presence of rat-liver S9 metabolic activation. The dose used was 30-fold that needed to induce a three fold increase in reversion in *S. typhimurium* strain TA100 (Koch *et al.* 1998).

Studies have been conducted with antimutagenic agents to determine the mechanism of IQ mutagenesis. In one of these studies, the use of theafulvins in a *Salmonella* assay (strain TA98 in the absence of metabolic activation) provided evidence that the inhibition of cytochrome P-450 by theafulvins correlated, in a concentration-dependent manner, with decreased mutagenic activity of IQ. Theafulvins were shown to inhibit *O*-dealkylation of methoxy-, ethoxy-, and pentoxy-resorufin (chemical probes for CYP1A2, CYP1A1, and CYP2B proteins, respectively, of the cytochrome P-450 system) and thereby inhibit the bioactivation of IQ to its mutagenic metabolites (Catterall *et al.* 1998).

Studies in which green tea or black tea was used in conjunction with IQ or its mutagenic metabolite (2-hydroxyamino-3-methylimidazo[4,5-*f*]quinoline, or *N*-hydroxy-IQ) in a *Salmonella* assay (strain TA98) in the absence of metabolic activation provided evidence that scavenging of *N*-hydroxy-IQ by the green tea or black tea caused a concentration-dependent inhibition of the mutagenic effect of IQ and its metabolite *N*-hydroxy-IQ on *Salmonella* (Chen and Yen 1997; Hernaez *et al.* 1998).

5.1.2 Induction of mutation in Escherichia coli

IQ caused prophage λ induction in *Escherichia coli* K12 in the presence of exogenous metabolic activation (Nagao *et al.* 1983, cited in IARC 1993).

The mutational specificity of IQ was evaluated in *E. coli* by testing the ability of IQ to inactivate the *URA3*-gene of yeast (*Saccharomyces cerevisiae*). The *URA3*-gene obtained from *S. cerevisiae* was randomly modified with *N*-hydroxy-IQ and transferred into *E. coli* (DB6656). Following incubation for one hour in a 5-fluoro-orotic acid (toxic to *URA3+*, selects for *URA3-* mutant clones), a spectrum of mutations was observed. These mutations included base-pair substitution (~70%), transversions, and transitions, as well as frameshift mutation, gene deletions and insertions, and gross alterations. More than 97% of the base-pair substitutions occurred at the GC pairs. In the *URA3* gene, the most common base substitution induced by IQ was found to be GC→AT transitions (52%), followed by GC→CG (25.9%) and GC→TA (18.5%) transversions (Broschard *et al.* 1998).

5.2 Plants

5.2.1 Chromosomal aberrations (CA)

5.2.1.1 Somatic mutation

A dose-dependent increase in the frequency of somatic mutations (yellow, dark green, and twin mutational spots on the leaves) was observed in the heterozygous strain T-219 of the soybean plant ($Y_{11}Y_{11}$) following treatment with IQ in the presence of exogenous metabolic activation. Preincubation of IQ with rat S9 liver homogenate increased the frequency of yellow spots by as much as 2 to 4 times. Treatment with the highest concentration (0.1 μg/mL) caused growth inhibition with deformed leaf development (Katoh *et al.* 1992).

5.3 Eukaryotic Systems

5.3.1 Mutagenicity in Drosophila melanogaster

5.3.1.1 Sex-linked recessive lethal assay

IQ induced sex-linked recessive lethal mutations in *Drosophila melanogaster* in two studies (Wild *et al.* 1985, Graf *et al.* 1992, both cited in IARC 1993).

5.3.1.2 Somatic mutation and recombination

IQ induced somatic mutations and recombination in *D. melanogaster* in two studies (Yoo *et al.* 1985, Graf *et al.* 1992, both cited in IARC 1993).

5.4 Mammalian Systems

5.4.1 In vitro *assays*

5.4.1.1 hprt *locus forward mutation test*

In two studies, IQ induced hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus forward mutations in Chinese hamster ovary (CHO) cells (uv5) in the presence of exogenous metabolic activation (Thompson *et al.* 1983, Brookman *et al.* 1985, both cited in IARC 1993). In two other studies, IQ induced gene mutations (gene locus unspecified) in Chinese hamster lung cells with exogenous metabolic activation (Nakayasu *et al.* 1983, Sugimura *et al.* 1989, both cited in IARC 1993).

In other studies, IQ did not increase the frequency of forward mutations at the adenine phosphoribosyl transferase (*aprt*) or *hprt* loci in CHO cells (*AA*8), in the presence of exogenous metabolic activation (Thompson *et al.* 1983, cited in IARC 1993). The mutation frequency at the *hprt* locus in Chinese hamster lung V79 cells was not increased following exposure to IQ (Loprieno *et al.* 1991, cited in IARC 1993). Similarly, IQ did not induce mutations in the *ouabain* locus in Chinese hamster lung cells in the presence of exogenous metabolic activation (Takayama and Tanaka 1983, cited in IARC 1993). *In vitro* exposure of human peripheral blood lymphocytes to IQ with exogenous metabolic activation also failed to induce forward mutations at the *hprt* locus (McManus *et al.* 1988b, cited in IARC 1993).

In more recent studies, treatment of CHO-K1 cells with IQ in the presence of exogenous S9 metabolic activation induced a significantly increased frequency of mutations at the *hprt* locus (Lee and Shih 1995).

In repair-deficient CHO (UV5P3) cells that express cytochrome P-450-1A2 and had been transfected with cDNA of either human *N*-acetyltransferase-2 or *Salmonella O*-acetyltransferase, IQ increased the incidence of mutations at the *aprt* locus about 3.1 x 10^3-fold, compared with the mutation rate in the parental CHO (UV5P3) cell line (Wu *et al.* 1997).

IQ induced mutations at the thymidine kinase and *hprt* loci in human lymphoblastoid cells, in the presence of exogenous rat-liver S9 metabolic activation. IQ significantly increased *hprt* mutant ion frequencies over those seen in untreated cells. This study also showed that the IQ metabolite IQ-nitrene was a ≥ 50-fold more potent a mutagen in human lymphoblastoid cells. IQ-nitrene is formed by the photoactivation of N3-IQ [an azido derivative (2-azido-3-methylimidazo[4,5-*f*]quinoline) of IQ] (Leong-Morgenthaler *et al.* 1998).

5.4.1.2 Genetic changes in animal tumor cells

Activated *c*-Ha-*ras* proto-oncogenes were found in four of seven IQ-induced Zymbal gland tumors in rats. The mutations were G→C transversions at the first base of codon 13 (two tumors), a G→T transversion at the second base of codon 13 (one tumor), and an A→T transversion at the second base of codon 61 (Kudo *et al*. 1991, cited in IARC 1993). Gene mutations also were found in the p53 gene in four of 15 IQ-induced Zymbal gland tumors in rats. These mutations were CGT→GGT, TGC→TTC, and GTG→TTG transversions, and GAA deletions at codons 156, 174, 214, and 256, respectively (Makino *et al*. 1992, cited in IARC 1993).

5.4.1.3 Chromosomal aberration tests

Chromosomal aberrations

Exposure of CHO cells to IQ in the presence of exogenous metabolic activation induced CA (Lopreino *et al.* 1991, cited in IARC 1993). Exposure of CHO (*uv5*) cells or CHO (AA8) cells to IQ did not induce an increased frequency of CA (Thompson *et al.* 1983, cited in IARC 1993).

Exposure of human peripheral blood lymphocytes to IQ in the presence of exogenous metabolic activation induced CA (Loprieno *et al.* 1991, cited in IARC 1993). However, negative results were obtained in another study using a higher IQ concentration in the presence of exogenous metabolic activation (Aeschbacher and Ruch 1989, cited in IARC 1993).

In other studies, IQ was evaluated for the ability to induce CA in Chinese hamster lung fibroblast (CHL/IU) cells. A significant increase in chromosomal aberrations was observed in cells exposed to IQ in the presence of metabolic activation. With metabolic activation, longer exposure to IQ significantly increased the frequency of aberrant cells only at 10 μg/mL. Only a weak induction of aberrations was observed at the highest concentration tested without metabolic activation (Miura *et al.* 1993). IQ did not induce CA in the CYP-deficient (V79-MZ and V79-NH) and XEMd-MZ cell lines. The results in the XEMd-NH cells were equivocal (Rodrigues *et al.* 1994).

Micronucleus test

IQ induced a small increase in micronucleus formation in human peripheral blood lymphocytes *in vitro* in the presence of exogenous metabolic activation (McManus *et al.* 1988b, cited in IARC 1993).

Exposure of ovine seminal vesicle cells with IQ in the presence of a prostaglandin H synthase metabolic activation system induced micronuclei in a statistically significant concentration-dependent manner (Degen *et al.* 1998).

5.4.1.4 Sister chromatid exchanges (SCEs)

An increased frequency of SCEs was observed in CHO (uv5) cells after exposure to IQ in the presence of exogenous metabolic activation. However, only a weak induction of SCEs was observed in CHO (AA8) cells exposed to IQ in the presence of exogenous metabolic activation (Thompson *et al.* 1983, cited in IARC 1993).

An increased frequency of SCEs was observed in human peripheral blood lymphocytes exposed to IQ in the presence but not in the absence of exogenous metabolic activation (Aeschbacher and Ruch 1989, cited in IARC 1993).

The genetically engineered V79 cell lines V79-MZ (CYP-deficient), V79-NH (CYP-deficient with endogenous acetyl transferase activity), XEMd-MZ (expressing rat CYP 1A2), and XEMd-NH (CYP 1A2 with endogenous acetyltransferase activity) were exposed to IQ at concentrations of 10, 30, and 90 μM in the absence of metabolic activation. SCEs were not increased in any cell line at any concentration tested (Rodrigues *et al.* 1994).

5.4.1.5 DNA damage/repair test

IQ induced DNA single-strand breaks in mouse hepatocytes without exogenous metabolic activation (Hayashi *et al.* 1985, cited in IARC 1993) and in radiation-induced mouse leukemic cells with exogenous metabolic activation (Caderni *et al.* 1983, cited in IARC 1993). IQ also induced DNA single-strand breaks in rat hepatocytes without exogenous metabolic activation (Caderni *et al.* 1983; Holme *et al.* 1987, both cited in IARC 1993).

IQ caused a significant concentration-dependent increase in the incidence of DNA strand breaks and percent tail DNA in cultured T-antigen immortalized human liver epithelial cells tested in the Comet assay (Barcelo *et al.* 1998). Human T5-neo (parental cell line) and T5-1A2 (human cytochrome CYP1A2-positive cell line) cells were exposed to IQ without exogenous metabolic activation. A significant dose-dependent increase of percent tail DNA was observed in the T5-

1A2 cells relative to untreated controls at all IQ concentrations tested. IQ did not induce DNA strand breaks in the T5-neo cells, although there was a slight increase in the percent tail DNA at the highest IQ concentration tested. IQ was not cytotoxic to the T5-neo and T5-1A2 cells.

5.4.1.6 Unscheduled DNA synthesis (UDS)

IQ induced UDS in both Syrian hamster and mouse hepatocytes *in vitro* in the absence of exogenous metabolic activation (Yoshimi *et al.* 1988, cited in IARC 1993). Exposure of primary rat hepatocytes to IQ without exogenous metabolic activation also induced UDS (Barnes and Weisburger 1985; Yoshimi *et al.* 1988, both cited in IARC 1993).

UDS induction was assessed in liver slices of rat, mouse, and human origin. Increased UDS was observed in both mouse and human cultured liver slices at the concentration ranges tested. Induction of UDS was not observed in the cultured rat liver slices (Beamand *et al.* 1998).

5.4.2 In vivo *assays*

5.4.2.1 Mouse spot test

A single intraperitoneal (i.p.) injection of IQ at a dose of 20 mg/kg body weight did not induce mutations in the mouse coat color spot test. A single *in utero* IQ dose of 400 mg/kg maternal body weight also failed to cause mutation in mouse melanocytes (Wild *et al.* 1985, cited in IARC 1993).

5.4.2.2 Host-mediated assay

IQ-induced mutagenicity was observed in two host-mediated assays with mice. In the first assay, a single i.p. dose of 0.198 mg/kg of IQ was administered to intrasanguinous NMRI mouse hosts to *S. typhimurium* strain TA98 (Wild *et al.* 1985, cited in IARC 1993). In the second assay, a single peroral dose of 2.3 mg/kg of IQ was administered to intrasanguinous Swiss albino mouse hosts to *E. coli* strains M343/753 and M343/765 (Knasmüller *et al.* 1992, cited in IARC 1993).

5.4.2.3 Chromosomal aberrations

A single IQ dose of 160 mg/kg administered i.p., did not increase the frequency of CA in the bone marrow cells of mice (Minkler and Carrano 1984, cited in IARC 1993). However, a single IQ dose of 50 mg/kg administered i.p. increased the frequency of CA in rat hepatocytes (Minkler and Carrano 1984, cited in IARC 1993).

5.4.2.4 DNA damage/repair test

Single-strand breaks

An IQ dose of 10 mg/kg (delivered via an unspecified route) induced DNA strand breaks in mouse liver cells (Hayashi *et al.* 1985, cited in IARC 1993).

Male CD-1 mice were given a single i.p. injection of IQ at a dose of 13 mg/kg. The stomach, liver, lung, kidney, brain, spleen, and bone marrow of the animals were examined at various time intervals (0, 1, 3, and 24 hours) after the injection. DNA damage (fragmentation) was observed in the liver, lung, and brain three hours after the injection and in the stomach and kidney 24 hours after the injection. The DNA damage observed in the liver and brain returned to control

levels at 24 hours, but persisted in the lungs. Significant DNA damage could be observed in the stomach and kidney at 24 hours but not at three hours (Sasaki *et al.* 1997).

Micronucleus test

IQ did not induce an increased frequency of micronuclei in mice administered a single i.p. injection at a dose of 594 mg/kg (Wild *et al.* 1985, cited in IARC 1993) or with a single peroral dose of 40 mg/kg (Loprieno *et al.* 1991, cited in IARC 1993).

5.4.2.5 Sister chromatid exchanges (SCEs)

IQ did not increase the frequencies of SCEs in bone marrow cells of mice administered a single i.p. injection dose of 20 mg/kg (Minkler and Carrano 1984, cited in IARC 1993) or in hepatocytes of rats administered a single i.p. injection at a dose of 50 mg/kg bw (Sawada *et al.* 1991, cited in IARC 1993).

5.5 Summary

IQ induces mutations in *S. typhimurium*, *E. coli*, and meristematic cells of soybean plants. IQ induces mutations and DNA damage in *D. melanogaster* and is both clastogenic and mutagenic in mammalian cells *in vitro* and *in vivo*. Mutations in c-Ha-*ras* and p53 genes were found in some IQ-induced Zymbal gland carcinomas in rats. Mammalian cells rich in cytochrome P-450-1A2 and *N*-acetyltransferase-2 are more susceptible to IQ induction of mutations, SCEs, CA, and DNA breaks, than cells not expressing these proteins.

6 Other Relevant Data

6.1 Absorption, distribution, metabolism, and elimination (ADME)

6.1.1 Absorption, distribution, and elimination

After oral administration (gavage or dietary) to rats, IQ is rapidly absorbed, primarily from the small intestine, metabolized, and excreted in the urine and feces (Sjödin and Jägerstad 1984, Alldrick and Rowland 1988, and Inamasu *et al.* 1989, all cited in IARC 1993).

Intravenously administered ^{14}C-labeled IQ was widely distributed in male NMRI, pregnant NMRI, and female C3H mice (Bergman 1985, cited in IARC 1993). Although IQ crossed the placental barrier, it did not accumulate or persist in fetal tissues.

6.1.2 Metabolism

Human liver microsomes activate IQ to DNA-reactive metabolites, including *N*-hydroxy-IQ; the isozyme involved has been tentatively identified as CYP1A2 (Butler *et al.* 1989, Shimada *et al.* 1989, and McManus *et al.* 1990, all cited in IARC 1993). In human fetal liver tissue, cytochrome P-450 HFLa is the main enzyme involved in activation of IQ (Kitada *et al.* 1990, cited in IARC 1993). IQ is oxidatively metabolized to *N*-hydroxy-IQ by rat and rabbit hepatic microsomal enzymes (Yamazoe *et al.* 1983, Kato 1986, and McManus *et al.* 1988a, all cited in IARC 1993). DNA-reactive IQ metabolites also were found in human mammary epithelial cells cultured in the presence of IQ (Pfau *et al.* 1992, cited in IARC 1993). IQ also is subject to oxidation via a prostaglandin hydroperoxide-dependent pathway present in microsomes isolated from ram seminal vesicles (Wild and Degen 1987, Petry *et al.* 1989, both cited in IARC 1993). Turesky *et al.* (1991, cited in IARC 1993) reported that cytosols from human liver and colon are able to catalyze the 7-hydroxylation of IQ. The hydroxy-IQ metabolite can be further esterified by *O*-acetyltransferase to *N*-acetoxy-IQ and sulfated by sulfotransferase to IQ-*N*-sulfate (Kato and Yamazoe 1987, cited in IARC 1993; Snyderwine *et al.* 1992a).

Another study reports that the initial step in IQ metabolism can be either *N*-hydroxylation or *N*-acetylation. In studies using monkey kidney COS cells transiently transfected with human CYP1A1, CYP1A2, and *N*-acetyltransferase (NAT)1 or NAT2, hydroxylation is catalyzed by either cytochrome P-450 isozyme (predominantly CYP1A1), while NAT1 or NAT2 carries out the *N*-acetylation reaction (NAT2 > NAT1). *N*-acetylated IQ can undergo *N*-hydroxylation (catalyzed by CYP1A1 or CYP1A2) to IQ-hydroxamic acid. The two pathways converge through *N*,*O*-transacetylation and *O*-acetylation, both of which yield *N*-acetoxy-IQ. It is presumed that the spontaneous degradation of *N*-acetoxy-IQ leads to the formation of an unstable nitrenium ion capable of binding to DNA (Figure 6-1) (Probst *et al.* 1992).

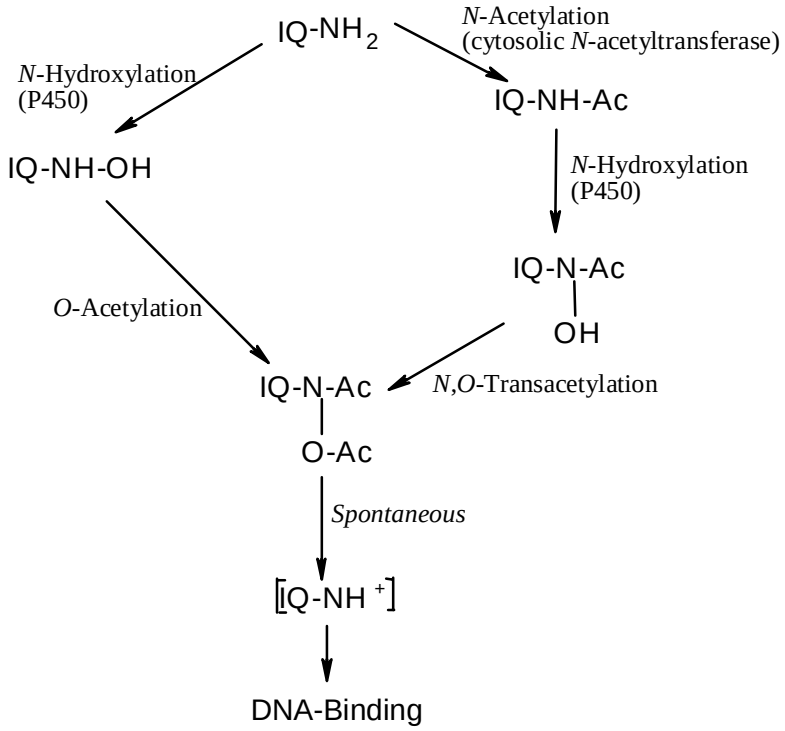

Figure 6-1. Metabolic activation of IQ

Source: Snyderwine *et al.* (1992b, 1995)

A recent study (Liu and Levy 1998) has confirmed the earlier findings that a prostaglandin hydroperoxide-dependent pathway can activate IQ to DNA-binding metabolites (Wild and Degen 1987, Petry *et al.* 1989, both cited in IARC 1993). Figure 6-2 presents the major routes of IQ metabolism in monkeys (Synderwine *et al.* 1992b, 1995).

In addition to *N*-oxidation, metabolites were formed from cytochrome P-450-mediated ring oxidation (at position C-5) and *N*-demethylation. Turesky *et al*. (1993) administered IQ to rats and isolated ring (C-5) oxidation metabolites. The glucuronide of *N*-hydroxy-IQ was detected in the urine of IQ-dosed rats (Turesky *et al.* 1993). *In vitro*, glucuronidation of 7-hydroxy-IQ was mediated by uridine 5′-diphosphoglucuronic acid-dependent glucuronyl transferase isolated from human and rat livers (Kaderlik *et al.* 1994).

Enteric bacterial flora from mice, rats, and humans also may contribute to the conversion of IQ to the directly mutagenic 7-hydroxy derivative (Bashir *et al.* 1987, Rumney *et al.* 1993a). Intestinal bacteria from rats and mice convert IQ to 7-hydroxy-IQ more rapidly than bacteria isolated from human fecal samples. In rats, consumption of diets supplemented with beef drippings increased the rates of enteric-bacteria-mediated conversion of IQ to 7-hydroxy-IQ, relative to low-fat diets (Rumney *et al.* 1993b).

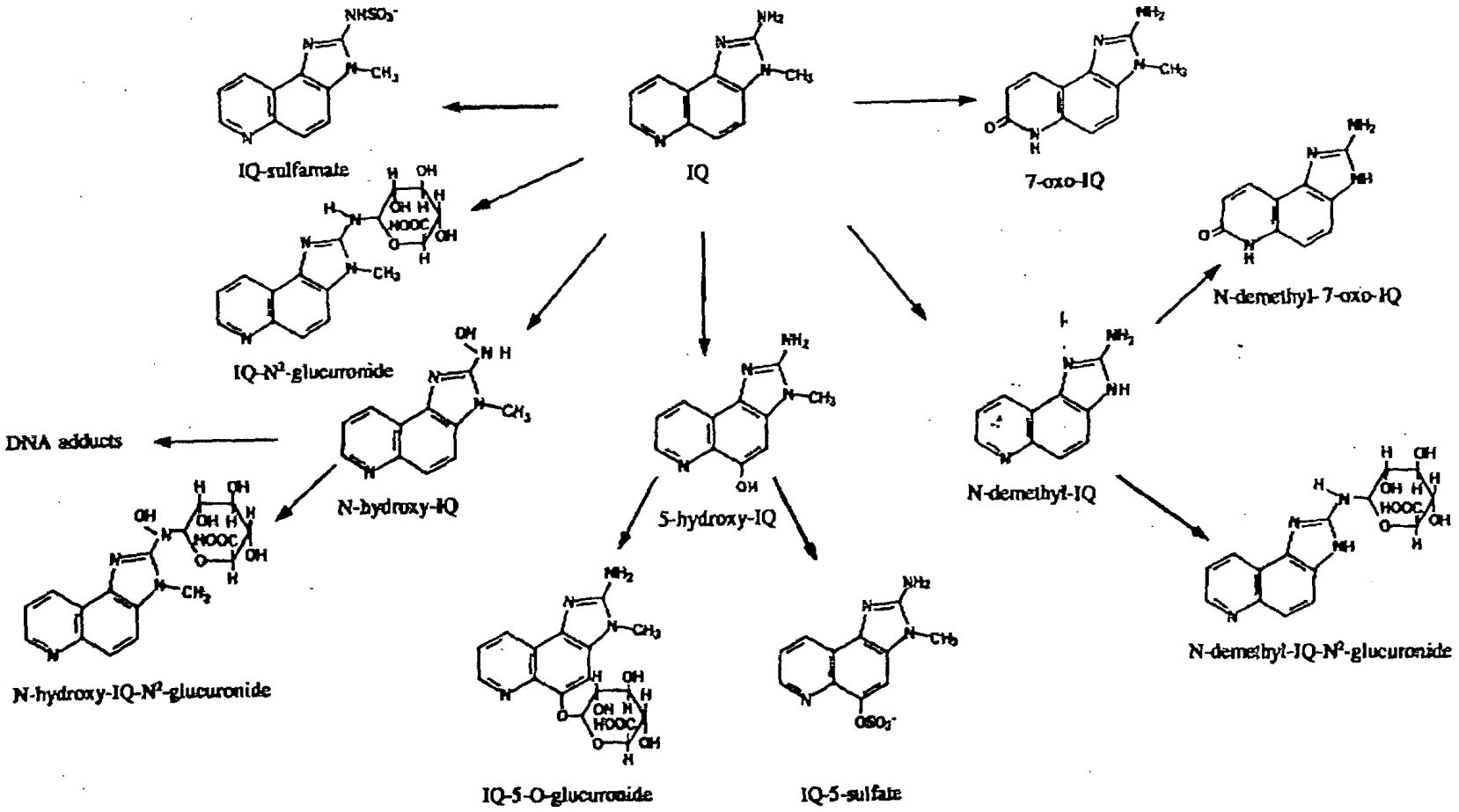

Figure 6-2. Major routes of IQ metabolism in the monkey

Source: Snyderwine *et al.* (1995)

6.2 Formation of DNA adducts

Metabolic activation of IQ via cytochrome P-450-mediated *N*-hydroxylation and subsequent esterification yields two reactive metabolites, *N*-hydroxy-IQ and *N*-acetoxy-IQ (Snyderwine *et al.* 1988a, 1988b, cited in IARC 1993). These reactive metabolites bind to DNA (Figure 6-3) (Snyderwine *et al.* 1988a, cited in IARC 1993). *N*-hydroxy-IQ binds nonenzymatically with polyguanylic acid *in vitro* (pH 7.4) in the presence of polynucleotides (Snyderwine *et al.* 1988a, 1988b, cited in IARC 1993).

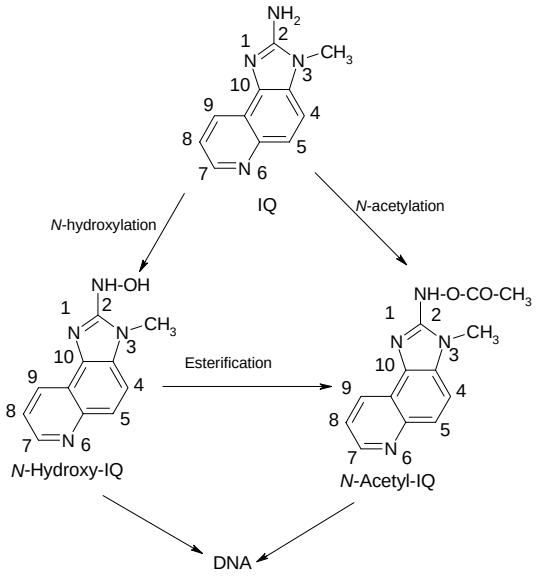

Figure 6-3. Structures of DNA-adduct-forming metabolites of IQ

Source: Snyderwine et al. 1988a, cited in IARC 1993)

IQ-DNA adducts were detected in bacteria exposed to IQ with exogenous metabolic activation by means of the ^{32}P-postlabeling method (Asan *et al.* 1987, cited in IARC 1993).

More recently, DNA adducts formed in *S. typhimurium* strain YG1024 (TA98 pYG219) (which overexpresses acetyltransferases) after treatment with IQ have been detected with the ^{32}P-postlabeling method. Exogenous metabolic activation was provided by prostaglandin H synthase (Probst *et al.* 1992, cited in Degen *et al.* 1998).

The ^{32}P-postlabeling method also has been used to detect IQ-DNA adducts in Syrian hamster embryo cells exposed to IQ in the presence of exogenous metabolic activation (Asan *et al.* 1987, cited in IARC 1993). IQ-DNA adducts also were detected in rat hepatocytes exposed to IQ at a concentration of 10 μg/mL in the absence of exogenous metabolic activation (Dirr *et al.* 1989, Wallin *et al.* 1992, both cited in IARC 1993).

In mammalian *in vivo* systems, the ^{32}P-postlabeling and ^{14}C-labeling methods have been used to detect formation of IQ-DNA adducts in multiple organs and species. Adducts were found in the liver and several other organs of rats following peroral and i.p.

administration of IQ at doses ranging from 5 mg/kg (Zu and Schut 1991a, 1991b, both cited in IARC 1993) to 100 mg/kg (Yamashita *et al.* 1988, cited in IARC 1993), in the liver and heart of rats following dietary administration of IQ at a dose of 36 mg/kg for 4 weeks (Övervik *et al.* 1991, cited in IARC 1993), in the liver and several other organs of mice after peroral and i.p. administration of IQ at doses ranging from 5 mg/kg (Zu and Schut 1991a, cited in IARC 1993) to 40 mg/kg (Loprieno *et al.* 1991, cited in IARC 1993), and in the liver of cynomologus monkeys following 15 peroral administrations of IQ at a dose of 20 mg/kg (Snyderwine *et al.* 1988c, cited in IARC 1993). The major product identified cochromatographs with *N*-(deoxyguanosi-8-yl)-IQ (Schut *et al.* 1991, cited in IARC 1993).

More recently, CDF_1 mice were administered IQ in the diet at a concentration of 0.01% for three weeks or by gavage at single doses of 50 mg/kg of IQ to test for the ability of IQ to form DNA adducts. During the 12 days of monitoring, IQ-DNA adducts formed from dietary IQ, and removal of adducts from the liver, lungs, and stomach was slow, with 40.8% to 64.5% of the day-1 adducts remaining on day 12. In contrast, IQ-DNA adducts formed after single gavage administration of IQ were removed from the liver and stomach much more rapidly; only 10.3% to 14.3% of day-1 adducts remained on day 8. The rate of removal of IQ-DNA adducts from the lungs was independent of the mode of IQ administration. The principal adduct in mammalian species, as detected by the ^{32}P-postlabeling method, is *N*-(deoxyguanosine-8-yl)-IQ (68.9% to 83.4%) (Schut *et al.* 1997a). IQ also forms a minor adduct with the exocylic amino group (N^2) of guanine. Although analysis with the ^{32}P-postlabeling method indicates that IQ forms additional minor adducts, the structures of these adducts have not been elucidated (Schut *et al.* 1997b).

Snyderwine *et al.* (1992b, 1997) reported ten (and identified nine) IQ metabolites in the urine, bile, and feces of monkeys following IQ administration in a cancer bioassay. The derivatives identified for IQ included both primary metabolites and their sulfate or β-glucuronic acid conjugates. Both *N*-hydroxyl-IQ and its glucuronide also have been detected in the urine of IQ-dosed monkeys. *N*-hydroxyl-IQ has been shown to form adducts with DNA in a number of organs, particularly the liver (a carcinogenesis target organ for IQ in monkeys), kidney, and heart (Snyderwine *et al.* 1992a).

IQ-DNA adducts are formed in human mammary epithelial cells incubated with IQ (Pfau *et al.* 1992, cited in IARC 1993). In more recent studies, the ^{32}P-postlabeling method was used to detect IQ-DNA adducts following *in vitro* IQ exposure of human mammary gland epithelial cells (Fan *et al.* 1995) and TK6 human lymphoblastoid cells (Leong-Morganthaler *et al.* 1998). IQ-DNA adducts also have been detected with the ^{32}P-postlabeling method in a variety of tissues in mice following oral (Davis *et al.* 1996; Schut *et al.* 1997a,b) or i.p. exposure (Nerurkar *et al.* 1995), in rats following oral exposure (Davis *et al.* 1994; Schut *et al.* 1994, 1997a; Turesky *et al.* 1996a, 1997; Turesky and Markovic 1995; Xu *et al.* 1996, 1997), and in nonhuman primates following oral exposure (Turesky *et al.* 1996b, 1997).

In a subsequent study, Stone *et al.* (1998) found that DNA adduct levels were consistently higher (>10-fold) in mammary epithelial cells after incubation with *N*-

hydroxy-IQ than after incubation with IQ *per se*. The results of these experiments indicate that *N*-hydroxylation may be the rate-limiting step in the activation of IQ to DNA-adduct-forming products. Since *N*-acetylation appears to be a metabolic step in activation of IQ, it was proposed that the formation of IQ-DNA adducts in human cells is influenced by acetylator phenotype (i.e., rapid or slow acetylation). When human mammary epithelial cells of known acetylator phenotype (NAT2) were incubated with IQ, the mean level of IQ-DNA adducts was higher in cells from rapid acetylator donors than in cells from slow acetylator donors. However, the difference in adduct level was not statistically significant, probably because of the large variability in adduct levels (Stone *et al.* 1998).

6.3 Summary

IQ is rapidly absorbed and distributed in mammalian systems following oral administration. The initial step in IQ metabolism is either *N*-hydroxylation or *N*-acetylation, mediated by cytochrome P-450 isozymes and *N*-acetyltransferase to yield *N*-acetoxy-IQ and *N*-hydroxy-IQ, respectively. These mutagenic metabolites bind covalently to DNA, forming DNA adducts *in vitro* and *in vivo*, inducing base-pair substitution, transversion, transition, frameshift, and deletion/insertion mutations. For the optimal metabolic activation of IQ, mammalian cells require the presence of cytochrome P-450-1A2 and *N*-acetyltransferase-2 to metabolize IQ into genotoxic forms that induce mutations, SCEs, CA, and DNA breaks. Cells not expressing these proteins are less sensitive to IQ genotoxicity/mutagenesis. *N*-acetoxy-IQ also may spontaneously degrade to form an unstable nitrenium ion capable of binding to DNA.

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Appendix A: IARC. (1993). *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines, and Mycotoxins.* Monographs of Evaluation of Carcinogenic Risks to Humans. IQ (2-Amino-3-methylimidazo[4,5-*f*]quinoline). Lyon, France. World Health Organization. Volume 56 pp. A-1—A-34.